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# Detection, occurrence and monthly variations of typical lipophilic marine toxins associated with diarrhetic shellfish poisoning in the coastal seawater of Qingdao City, China

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## HIGHLIGHTS

- A new method was developed for the determination of OA, YTX, and PTX2 in seawater.
- OA and PTX2 commonly existed in the seawater along the coastline of Qingdao City.
- OA and PTX2 were present almost every month in the coastal seawater of Qingdao.
- The highest concentrations of OA and PTX2 occurred in August and July respectively.

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## ABSTRACT

In recent years, related research has mainly examined lipophilic marine toxins (LMTs) in contaminated bivalves or toxic algae, whereas the levels of LMTs in seawater remain largely unexplored. Okadaic acid (OA), yessotoxin (YTX), and pectenotoxin-2 (PTX2) are three typical LMTs produced by certain marine algae that are closely linked to diarrhetic shellfish poisoning. In this study, a new method of solid phase extraction combined with liquid chromatography – electrospray ionization ion trap tandem mass spectrometry was developed to determine the presence of OA, YTX, and PTX2 in seawater simultaneously. Satisfactory sensitivity, repeatability (RSD < 25.00%) and recovery (56.25–70.18%) of the method were achieved. Then, the method was applied to determine the amounts of the three toxins in the coastal seawater. OA and PTX2 were detected in all the seawater samples collected from eight locations along the coastline of Qingdao City, China on October 23, 2012, with concentration ranges of OA 4.24–9.64 ng $L^{-1}$ and PTX2 0.42–0.74 ng $L^{-1}$. Monthly concentrations of OA and PTX2 in the seawater of four locations were determined over the course of a year, with concentration ranges of OA 1.41–89.52 ng $L^{-1}$ and PTX2 below detectable limit to 1.70 ng $L^{-1}$. The peak values of OA and PTX2 in coastal seawater were observed in August and July, respectively. Our results suggest that follow-up research on the fate modeling and risk assessment of LMTs in coastal seawater should be implemented.



## 1. Introduction

Lipophilic marine toxins (LMTs) are secondary metabolites produced by marine algae. These toxins can be accumulated in bivalves via feeding behavior (Hess, 2010). Although some toxins have no adverse effects on shellfish themselves, severe intoxication may occur if humans consume the contaminated shellfish, with diarrhea, nausea and emesis as the common symptoms (Quiliam and Wright, 1995; Gerssen et al., 2010; Pfannkuchen et al., 2012). Okadaic acid (OA), yessotoxin (YTX), and pectenotoxin-2 (PTX2) are typical LMTs found in bivalves or algae because of diarrhetic shellfish poisoning (DSP) (Yasumoto et al., 1978; Yasumoto et al., 1985; Murata et al., 1987; Lee et al., 1989). Fig. S1 (Supplementary Material) illustrates the structures of these toxins. OA and its analogs or derivatives have been proven to be the main cause of DSP incidents (Vale and Sampayo, 2002). The OA class of

*Abbreviations:* DSP, diarrhetic shellfish poisoning; LC-ESI/IT-MS/MS, liquid chromatography– electrospray ionization ion trap tandem mass spectrometry; LMTs, lipophilic marine toxins; MRM, multiple reaction monitoring; OA, okadaic acid; PTX2, pectenotoxin-2; SPE, solid phase extraction; YTX, yessotoxin.

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compounds are also believed to be tumor promoters (Suganuma et al., 1988; Fujiki and Suganuma, 1993), with the consumption of contaminated shellfish possibly increasing the risk of cancers in the digestive system (Cordier et al., 2000; Manerio et al., 2008). No human intoxications caused by YTX, PTX2 and their derivatives have been reported. However, the LD50 of YTX equivalents for mice is 750 μg $kg^{-1}$ when injected i.p., and oral administration of YTX equivalents results in the tumidness of some of the heart muscle cells of mice (Aune et al., 2002). Both oral administration and i.p. injection of PTX2 equivalents can cause damage to the livers of mice (Miles et al., 2004; Espiña and Rubiolo, 2008). The permitted levels of OA, YTX, and PTX2 equivalents in shellfish were proposed by the European Food Safety Authority (Alexander et al., 2008a, b, 2009).

In the past decades, much effort has been focused on research on LMTs in contaminated biovalves and toxic algae (Franchini et al., 2009; Bovee et al., 2011; Gerssen et al., 2011; Visciano et al., 2013). LMTs in marine environments have also gained increasing attention in recent years (Mendoza et al., 2008; Hitchcock et al., 2012), and OA, YTX, and PTX2 have often been detected in seawater (Lane et al., 2010). PTX2 has been reported in the seawater of Jiaozhou Bay (Li et al., 2010), which is near Qingdao City. In previous research, passive samplers with macroporous resin were used to enrich LMTs in seawater to determining their presence (Fux et al., 2009; Rundberget et al., 2009; MacKenzie, 2010). However, passive samplers cannot contribute to determining the true levels of the toxins or their exact variations in seawater. Besides, the adsorption procedure of passive samplers can be easily influenced by the circumstances involved and by weather situations. Furthermore, in situ trials are time consuming. Consequently, the pollutant characteristics of LMTs in various seawaters and the ecological risks of these LMTs can hardly be assessed precisely. Establishing a fast and precise method to detect LMTs in seawater is therefore significant for both human health and marine environment protection.

The multiple reaction monitoring (MRM) mode of tandem mass spectrometry (MS/MS) has better sensitivity than the full-scan mode of conventional MS. In recent years, liquid chromatography (LC) coupled with tandem mass spectrometry has been widely used to detect multiple contaminants in various waters (Berset et al., 2010; López-Serna et al., 2011; Berset and Ochsenbein, 2012; Yuan et al., 2012; Boix et al., 2013; Gorga et al., 2013). LC-MS/MS has also been effectively used to detect LMTs in shellfish and algae (These et al., 2009; Gerssen et al., 2009b; These et al., 2011). Solid phase extraction (SPE) is a well established pretreatment method that is often used to enrich trace organic contaminants in water samples (Rodil et al., 2009; Wille et al., 2010); the approach has a good chance of recovery and repeatability. It has also been used for sample clean-up and analyte enrichment before LC-MS/MS analysis of LMTs in shellfish (These et al., 2009; Gerssen et al., 2009a). To the best of our knowledge, no previous research has focused on the use of commercially available SPE cartridges to accumulate LMTs in seawater samples.

In this research, liquid chromatography – electrospray ionization ion trap tandem mass spectrometry (LC-ESI/IT-MS/MS) combined with offline SPE was used to detect concentrations of three typical LMTs: OA, YTX, and PTX2 in seawater. The occurrence and monthly variations of these toxins in coastal seawater along the coastline of Qingdao City were also analyzed.

## 2. Materials and methods

### 2.1. Chemicals

Water used for sample pretreatment and LC-MS/MS analysis was deionized by a Milli-Q water purification system (Millipore, Bedford, MA, USA). Acetonitrile and methanol were both HPLC grade and purchased from Merck (Darmstadt, Germany). Ammonium hydroxide (⩾ 25%) of MS grade was purchased from Fluka (St. Louis, MO, USA). Standards of OA, YTX, and PTX2 were purchased from the National Research Council, Institute for Marine Biosciences (Halifax, Nova Scotia, Canada).

Primary stock solutions of OA, YTX, and PTX2 were prepared by dilution of the standards with methanol, with concentrations of 684.3 μg $L^{-1}$, 263.0 μg $L^{-1}$ and 429.5 μg $L^{-1}$, respectively. Mixed stock solutions were mixtures of OA, YTX, and PTX2 stock solutions in certain proportions. Mixed standard solutions were prepared by dilution of the mixed stock solutions with methanol. For instance, mixed standard solution A was a mixture of OA, YTX, and PTX2 at concentrations of 68.4 μg $L^{-1}$, 78.9 μg $L^{-1}$ and 12.9 μg $L^{-1}$, respectively. All solutions were stored at – 20 °C in the dark.

### 2.2. Sample collection

All seawater samples were collected from eight locations along the coastline of Qingdao City, Shandong Province, China (Fig. 1), with a sampling volume of 2 L in each location. Each seawater sample was a mixture of seawater from three sampling sites in one location, with certain spacing distances to ensure representativeness. The samples were preserved in closed, plastic buckets at 4 °C and then pretreated within 48 h. To verify the presence of OA, YTX, and PTX2 in the coastal seawater of Qingdao City, the sampling was conducted in eight locations on October 23, 2012. From November 2012 to September 2013, sampling was conducted in four locations (L1: Zhongyuan Wharf; L4: No. 3 Bathing Beach; L6: Mai Island; L8: Shilaoren Beach) around the 23rd of every month to study the monthly variations in OA, YTX, and PTX2 in the coastal seawater of Qingdao City.

### 2.3. Sample pre-treatment

All seawater samples were filtered by 0.45 μm glass microfiber filters (GF/A, Whatman Schleicher & Schuell, Maidstone, England) to remove the visible particulate matter and possible toxic algae. Then, SPE was conducted with Oasis HLB cartridges (200 mg, 6 mL, Waters, Medford, MA, USA). After the cartridges were preconditioned with 3 mL methanol and then 3 mL deionized water, 200 mL of seawater was loaded into each cartridge at a flow rate of 1 mL $min^{-1}$. Then, the cartridges were rinsed with 3 mL methanol/water (15:85, v:v) and dried under vacuum for about 5 min. The extracts were achieved by elution of the cartridge with 3 mL ammonium hydroxide/methanol (1:99, v:v) 3 times. Subsequently, the extracts were evaporated by a rotary evaporator (Eyela, Tokyo, Japan) until dry, with the water temperature at 40 °C, reconstituted with 1 mL methanol, and filtered by a 0.22 μm membrane filter before the extracts were transferred to an analysis vial. Other Strata-X cartridges (100 mg, 6 mL, Phenomenex, Torrance, CA, USA) were also used in preliminary experiments with the same procedures as described above.

Seawater samples collected from the Arctic Ocean were used as blank samples. Spiked seawater sample was prepared by addition of 100 μL mixed standard solution A to a 200 mL blank seawater sample and by thorough mixing; the concentration was OA 34.2 ng $L^{-1}$, YTX 39.45 ng $L^{-1}$, and PTX2 6.45 ng $L^{-1}$.

### 2.4. HPLC–MS/MS analysis

A 1200 series HPLC system (Agilent Technologies, Wilmington, DE, USA) consisting of a vacuum degasser, a quaternary pump, and a autosampler and equipped with a ZORBAX Extend-C18 analytical column (3 mm × 150 mm, particle size: 3.5 μm, Agilent Technologies, Wilmington, DE, USA) was used in chromatographic